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A METHOD FOR DETERMINING THE BACTERICIDAL ACTIVITY OF GASEOUS DISINFECTANTS

[Following is the translation of an article by V. T. Osipyan and N. D. Uspenskiy, Military-Medical Order of Lenin Academy imeni S. M. Kirova, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 8, 1964, pages 8-12. It was submitted on 11 Apr 1963. Translation performed by Sp/7 Charles T. Ostertag Jr.]

In recent years gaseous disinfectants have been used more and more for disinfection and sterilization -- ethylene oxide, a mixture of ethylene oxide with carbon dioxide (Carboxide, Oxyfume-20), with Freon (Cryoxide) and methyl bromide. However, the choice of gases possessing disinfection properties is extremely limited. At the same time the gaseous method of disinfection is acquiring all the greater importance, since it is the only promising method for the mass disinfection of a number of objects of the external medium which cannot be disinfected with the help of damp and chamber disinfection. Besides this, the gaseous method is simple in application and cheaper than the other methods. This also explains the increasing interest by disinfection personnel in such fumigants.

The promising aspects of the gaseous method of disinfection and the growing needs of practice are creating the necessity for the study and selection of the most effective gaseous substances. The cited problem can be successfully resolved if the disinfection personnel were armed with a method for a comparative evaluation of the effectiveness of new gases with those already known. However, at the present there is a satisfactory method only for the characteristics of liquid disinfectants. There is no such method for determining the effectiveness of gaseous substances. The stated provision is the reason that at the present time investigators in the Soviet Union and abroad use diverse methodical procedures when studying the bactericidal activity of gases.

A comparative study of the bactericidal activity of formaldehyde and sulfurous acid anhydride was carried out by Ilkevich (1905), chloropicrin, chlorine and carbon disulfide by Semikoz et al. (1927), chloropicrin, methyl bromide, hydrocyanic acid and formaldehyde by Sayki (1952), methyl bromide and hydrobromic acid by Kolb et al (1955), ethylene oxide, methyl bromide and formaldehyde by Pan et al. (1961). An analysis of the stated works shows that certain authors conducted their experiments in glass

beakers with a suspension of bacteria as tests, others in chambers and autoclaves with pieces of tissue that were impregnated in a bacterial suspension, and still others in accommodations with an arrangement of bacterial test objects in the materials being treated. Therefore the results of checking the disinfection activity of gases obtained by various methodical procedures were contradictory and didn't permit an objective evaluation of their effectiveness and the rationality of utilization.

What has been said fully concerns methyl bromide also. In literature there is no common opinion concerning its disinfecting activity. Some investigators (Reddish, 1954; Sayki, 1952) consider methyl bromide as a weakly active disinfectant, while other investigators (Kolb et al., 1952, Subbotin, 1962, and others) consider it highly effective with a broad spectrum of bactericidal action and recommend it for disinfecting objects infected with anthrax spores.

The absence of a common method for determining the degree of disinfection activity of methyl bromide led to the fact that investigators proposed various standards for using this preparation for the disinfection of objects infected with the spore forms of microbes. Thus, for obtaining a complete disinfecting effect, Kolb et al (1952) suggested methyl bromide in a concentration of 3.4 - 3.9 g/m^3 for an 18 hour exposure, Subbotin (1962) -- 2000 g/m^3 for 24 hours, Kulish and Amelina (1961) -- 1200 g/m^3 for two hours, and Likhacheva (1962) -- 3810 g/m^3 for 72 hours at 30°.

The lack of a common approach to an evaluation of gaseous disinfectants promotes the necessity for developing a method which would make it possible to determine the level of antibacterial activity of fumigants and to select the most effective preparations out of these.

It is known that the bactericidal activity of a disinfectant is determined by comparing it with a generally accepted standard. Thus, for example, for solutions of cyclic organic compounds, phenol serves as the standard, and for chlorine-containing preparations -- chloramine. There is no standard for the characteristics of gaseous disinfectants. Therefore, when studying the bactericidal activity of methyl bromide we compare its activity with the activity of ethylene oxide, the disinfecting properties of which have been studied sufficiently for the last 15-20 years.

We undertook the task of developing a method for the comparative evaluation of the bactericidal activity of gases which would meet specific requirements. The method should be accurate, make it possible to compare the bactericidal activity of various gases under similar test conditions (concentration, exposure, temperature, humidity and pressure) and ensure the obtaining of reproducible results. It should also be simple and practicable for ordinary laboratories at disinfection stations.

We determined the bactericidal activity of methyl bromide by an original method developed by us. It consisted of the following.

The tests were conducted in two 20 liter glass bottles which were hermetically sealed by rubber stoppers with notches in them for two taps (fig. 1). One tap was connected with a mercury manometer and the other with a vacuum pump. Coarse calico sacks with the experimental test-objects were hung to the stoppers of the bottles, in which, with the help of a pump, a vacuum was created, corresponding to the calculated quantity of the gases being tested. After this, gas was introduced into the bottles separately for obtaining a normal pressure in them. The control test-objects were not subjected to the action of the gas, but in other respects they were found in equal conditions with the test-objects. Test objects, 5 x 5 cm in size, were infected with E. coli or Staphylococcus aureus, which in resistance to temperature and phenol met the requirements of the instructions.

The creation of a vacuum in the bottles made it possible to guarantee a high degree of exactness in the amount of gas admitted into them. The magnitude of the vacuum was determined by using the Klaiperon-Mendeleev formula:

$$P = nRT\frac{1}{v};$$

where P -- partial gas pressure in the bottle, expressed in atmospheres, n -- number of grammols in the volume of the bottle, R -- gaseous constant equal to 0.08205, T -- absolute temperature (273.2 + t, where t is the temperature of the medium at the moment of conducting the test), V -- volume of gas equal to the volume of the bottle.

It is known that the concentration of any gas C is equal to $\frac{n}{v}$ grammols. Placing the value C in the Klaiperon-Mendeleev formula, we obtain $R = P \cdot T \cdot C$, from where $C = \frac{P}{RT}$.

The number of grammols for 1 m³ is equal to $1000 \cdot C$, from where the quantity of grams of gas for 1 m³ comprises $1000 \cdot C \cdot M$, where M is the molecular weight of the gas.

Having designated the given quantity of gas in g/m³ as σ, we obtain $\sigma = 1000 \cdot C \cdot M$. Placing the value σ in the formula $C = \frac{P}{RT}$, we find that $\sigma = \frac{1000 \cdot P}{RT} \cdot M$, from where $P = \frac{R \cdot T\sigma}{1000 \cdot M}$, or

$$P = \frac{0.08205 \cdot (273.2 + t) \cdot \sigma \cdot 760}{1000 \cdot M}.$$

We will present an example of a calculation for the partial pressure of 10 g of ethylene oxide at 18.5°.

$$P = \frac{0.08205 \cdot (273.2 + t) \cdot 10 \cdot 760}{1000 \cdot 44.05}.$$

As a result we obtain P = 4.1 mm mercury column, and since the partial pressure corresponds to the vacuum, then for the introduction of

10 g of ethylene oxide into the bottle it is necessary to preliminarily create a pressure in it equal to 4.1 mm mercury column or 55.76 mm water gage.

A calculation applicable to 10 g of methyl bromide shows that the vacuum in the bottle should comprise 1.9 mm mercury column or 25.84 mm water gage.

$$p = \frac{0.08205 \cdot (273.2 + 18.5) \cdot 10 \cdot 760}{1000 \cdot 94.95}.$$

Upon completion of exposure to the action of both gases (30, 60, 90 and 120 minutes) the test objects were taken from the bottles and beaten with beads for 15 minutes in sterile tap water, after which an 0.1 ml washing was sown on agar for the purpose of making a quantitative calculation of live microbes. The percentage of death of the microbes served as the indicator of the bactericidal activity of the given concentration of gas being tested.

As a result of the investigation conducted, data was obtained which made it possible to objectively characterize the degree of bactericidal activity of methyl bromide in comparison with ethylene oxide. In the tests, conducted under strictly identical conditions (gas concentration, temperature, pressure, humidity and exposure), it was established that ethylene oxide and methyl bromide exerted a different bactericidal effect on E. coli and Staphylococcus aureus. Thus, the influence of ethylene oxide on E. coli for 30, 60, 90 and 120 minutes with the dose of the preparation equal to 210 g/m^3, led to the death of 46.4, 91.8, 99.4 and 100% of the microbes correspondingly (fig. 2). The dying off of E. coli from the action of methyl bromide in the same periods of time was characterized by the following figures correspondingly: 40.4, 58.9, 61.3 and 69.3%.

Thus, in comparing the death curves of E. coli under the influence of ethylene oxide and methyl bromide, it can be seen that the bactericidal activity of ethylene oxide is considerably higher than methyl bromide. A difference in the bactericidal activity of the stated gases was observed in all the periods of time, but was especially sharply expressed under the influence for a period of 60 minutes. While under the influence of methyl bromide the death of E. coli reached 58.9%, while under these same test conditions with ethylene oxide 91.8% of the microbes died. With a two hour exposure, ethylene oxide ensured 100% death of E. coli, but the death of the microbes from the action of methyl bromide did not exceed 69.3%.

Somewhat different results were obtained when studying the antibacterial action of ethylene oxide and methyl bromide in respect to

Staphylococcus aureus. However, in this series of tests also, the effectiveness of ethylene oxide was greater than the effectiveness of methyl bromide. With an exposure equal to 60, 90 and 120 minutes, the percentage of death of Staphylococcus aureus from the influence of ethylene oxide exceeded the percentage of death of the microbes from the action of methyl bromide by 1.5, 2 and 1.2 times correspondingly (fig. 3).

The data obtained testified that the bactericidal activity of ethylene oxide considerably surpassed the activity of methyl bromide.

With the help of the method developed by us, it is possible to determine the comparative resistance of various microbes in respect to a given concrete gaseous disinfectant. Thus, in comparing the death curves of Staphylococcus aureus and E. coli (see figures 2 and 3), it is not difficult to notice that in the first the resistance to ethylene oxide is considerably higher than in the second. Such a difference in the stability of the stated microbes is particularly sharply noted with the influence of ethylene oxide in the first 30-60 minutes. With a 30 minute exposure the percentage of death of E. coli exceeded the percentage of death of Staphylococcus aureus by more than 4 times, and with a 60 minute exposure by 1.8 times. With a more prolonged influence (2 hours) ethylene oxide caused the 100% death of E. coli, while in the same time under the same test conditions the death of Staphylococcus aureus did not exceed 91%.

The resistance of Staphylococcus aureus to methyl bromide was considerably greater than the stability of E. coli, which was particularly demonstratively manifested at 30-60 minutes exposures: The percentage of death of E. coli was higher than the death of Staphylococcus aureus by 2.3 and 1.8 times correspondingly.

Thus, the new method developed by us made it possible to characterize the bactericidal activity of two and more gases under strictly identical test conditions.

Conclusions

1. A method has been developed for determining the comparative bactericidal activity of gaseous substances which makes it possible to characterize the bactericidal capability of gas-fumigants. The method is distinguished by great accuracy and a constant reproducibility of results.

2. Based on its bactericidal properties, methyl bromide is considerably inferior to the bactericidal activity of ethylene oxide.

3. Resistance to ethylene oxide and methyl bromide by Staphylococcus aureus is considerably greater than the resistance of E. coli.

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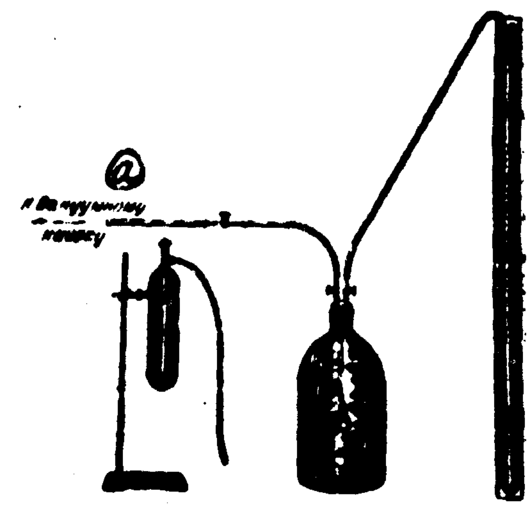

Figure 1. Unit for determining the bactericidal activity of gaseous disinfectants. a. - to the vacuum pump.

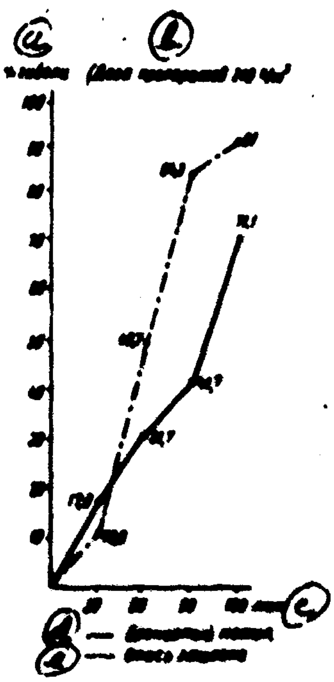

Figure 2. Bactericidal effect of ethylene oxide and methyl bromide on Staphylococcus aureus.

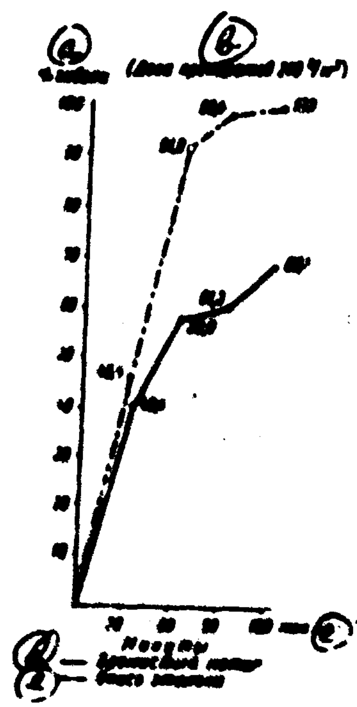

Figure 3. Bactericidal effect of ethylene oxide and methyl bromide on E. coli.

a. % death; b. (Dose of preparation 210 g/m^3); c. minutes; d. methyl bromide; e. ethylene oxide.